THE BLOCKING OF EXCYSTMENT REACTIONS OF COLPODA DUODENARIA BY ABSENCE OF OXYGEN [1]

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The encysted state of a protozoön may be considered one of high stability in that little or no energy is required for its maintenance. Cells in this condition are not in any dynamic equilibrium of diverse reactions, but in a static state. This investigation is concerned with the fundamental problems of the nature of the changes from the dynamic to the static and from the static to the dynamic state as found in the encystment and excystment of protozoa.

The excystment process of the holotrichous ciliate, *Colpoda duodenaria,* is more than a reactivation of metabolic enzyme systems. The process involves a redifferentiation of protoplasmic structures, cilia, etc., along with special physical-chemical systems such as the contractile vacuole system and in addition involves the processes for escape from the cyst membranes.

Though like all ontogenetic processes, the excystment process is thus complex, it may prove amenable to analysis since the encysted organisms may be made very nearly uniform and will remain in a resting state with little or no change until reception of an excystment-inducing substance from their environment. The uniformity of the cyst preparation is obtainable since encystment as well as excystment depends on environmental conditions which may be controlled (Taylor and Strickland, 1938). A standardized, constant biological material may thus be made available for an extended series of experiments, and quantitative as well as qualitative results compared throughout the series.

The investigation into the nature of the physiological processes involved in excystment has been (1) by chemical analysis of substances which will induce the process (see Haagen-Smit and Thimann, 1938)

[1] This study comprises part of the Ph.D. dissertation (Brown, 1938*a*) and has been briefly reported at the Richmond meetings of the A. A. A. S. (Brown, 1938*b*). Equipment used throughout this research was made available directly through the courtesy of Dr. C. V. Taylor and indirectly through a grant from the Rockefeller Foundation to Stanford University for Dr. Taylor's research in chemophysical biology.

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and (2) by determination of the relations between the excystment time (the time elapsed between substitution of the excystment solution for the salt solution in which the organisms are kept and emergence from the cyst membranes) and controlled environmental factors such as concentration of the excystment solution, temperature, oxygen tension, and x-ray irradiation (see Taylor, Brown, and Strickland, 1936; Brown and Taylor, 1938; and Brown, 1938*a*). This report presents the experimental data obtained in the study of oxygen tension as a limiting factor in excystment together with a further analysis of the physical-chemical processes of excystment of *Colpoda*.

Experimental

The *Colpoda* used in these experiments were carefully cultured and selected as to interfission age, then encysted in grooves in cellophane. The cyst-cellophane preparation was then thoroughly washed and then kept in a continuously flowing, dilute, balanced salt solution. The technique of making this preparation was developed by Mr. Strickland (Taylor and Strickland, 1935) and the cysts used throughout this study were prepared by him.

The time for excystment was determined by counting the number of still encysted organisms (100 to 150 at start of each test) at intervals throughout the period of emergence from the cyst membranes. The geometric mean time was then evaluated by graphical methods as described by Brown and Taylor (1938). This geometric mean time which is equal to the median excystment time is referred to throughout this paper as excystment time.

In each experiment a series of concentrations of excystment solution, Difco yeast extract, was used. This enables one to separate the excystment processes into two periods: (1) that inversely proportional to the concentration of the excystment solution, and (2) that independent of the concentration of the excystment solution (Brown and Taylor, 1938).

The control of oxygen tension necessitated the design and construction of a special airtight excystment chamber through which gases of various composition could be passed. This chamber must be mounted on a mechanical microscope stage and fitted to a microscope of approximately 150 × magnification. The final design (Fig. 1) was the result of a long series of improvements of chambers and mechanical stages. The chamber will contain a set of six Columbia dishes which can be successively observed by rotation of the glass plate forming the floor of the chamber. The upper, stationary part is made from a large Petri dish cover which is ground into the plate. This Petri dish cover is drilled at four points, a large hole, shown in the figure through which the

microscope objective projects, and three small ones, one for the inflow of the gas mixture, one for its outflow into a ⅛-inch tube about a foot long, and one, normally sealed and close to the gas outlet tube, through which twice concentrated excystment solutions, previously brought to equilibrium with the oxygen tension being tested, could be added to equal amounts of salt solution in the excystment dishes with a negligible admixture of air. The joint between the cover and floor and all joints about the objective have been sealed with paraffin oil throughout each experiment.

In the first series of experiments, the partial pressure of oxygen was reduced to $\frac{1}{10}$ that of air (from 150 mm. Hg to that of tank nitrogen,

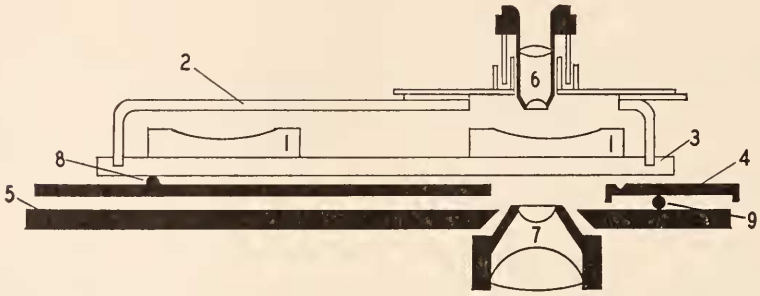

Fig. 1. Chamber and mechanical stage for studies of excystment of *Colpoda*. (1) Columbia dish containing cyst-cellophane preparation. (2) Glass cover of excystment chamber made from large, 150 mm., Petri dish. (3) Circular glass plate forming floor of chamber. (4) Moveable part of stage attached to a standard mechanical stage. (5) Microscope stage (specially constructed). (6) Microscope objective (10 × Zeiss, small size). (7) Microscope condenser. (8) One of three ball bearings in groove in moveable stage which support plate 3 and permit easy rotation of dishes. (9) One of three ball bearings between moveable stage and fixed stage.

approximately 15 mm. Hg for the tank used). The control experiments were identical in all respects with those with reduced oxygen tension except that air was flowing through the excystment chamber instead of the tank nitrogen. No differences of any kind were found when the excystment under an oxygen tension $\frac{1}{10}$ that of air was compared with the controls. A typical experimental run and two controls are shown in Figure 2. These results are in agreement with studies of respiratory rate under low oxygen tension for the free-swimming stages of the ciliates *Paramecium* (Lund, 1918, and Amberson, 1928) and *Colpoda* (Adolph, 1929); and further indicate that in none of the preceding work involving measurement of excystment time in solutions in contact with air was oxygen ever a limiting factor.

To obtain lower oxygen tensions, the nitrogen was purified by

bubbling through an acid chromous sulphate solution (M/10 Cr^{++}; pH = 2). The reduced state of the solution was maintained by the presence of amalgamated zinc prepared according to the methods of Stone and Beeson (1936). Gas exchange with the solution was facilitated by use of a sintered glass bubbler which broke up the gas stream into very small bubbles. After the air was washed out of the excystment chamber with this nitrogen and sufficient time elapsed so that equilibrium between the gaseous and liquid phase was approached, then excystment solution which had been de-oxygenated was added. There

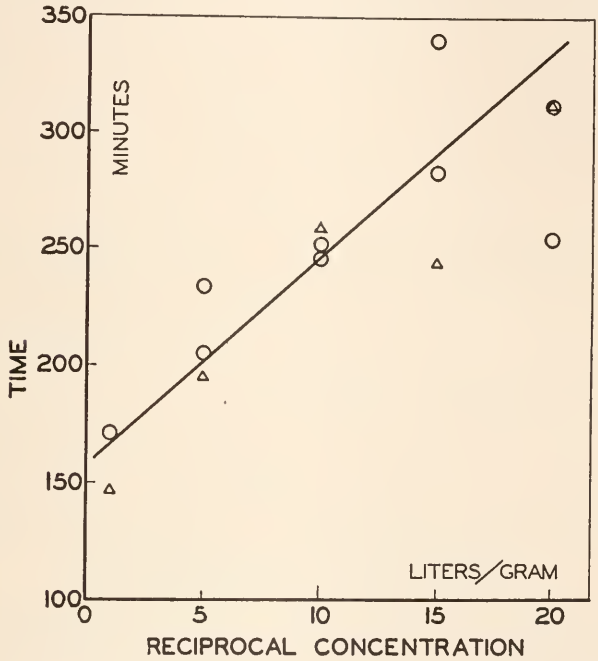

FIG. 2. Excystment of *Colpoda* at 20° C. ○, two control series; △, oxygen tension = 15 mm. Hg.

were no signs of excystment for periods of as long as 25 hours, though at 15 mm. Hg partial oxygen pressure, excystment at 20° C. would have been completed in two to three hours.

The data demonstrate much more than just a prevention of excystment by absence of oxygen, for upon admittance of air, normal excystment ensued; and further, the excystment time following the block was found to be independent of the duration of the block, independent of the concentration of the excystment solution, and equal in length to the period that was found from studies on relation between concentration

and excystment time to be independent of the concentration of the excystment solution. The experimental data for two of the series of experiments at 20° C. are shown in Figure 3. Detailed tables of these data can be found in Brown (1938*a*).

Apparently the reactions of the period in excystment depending on concentration of the excystment solution go to completion and the reactions of the subsequent periods are completely blocked in the absence of oxygen. These results are in agreement with the hypotheses previously made that the first period is controlled by a diffusion phenomenon and that the following periods at 20° C. are controlled by a reaction of

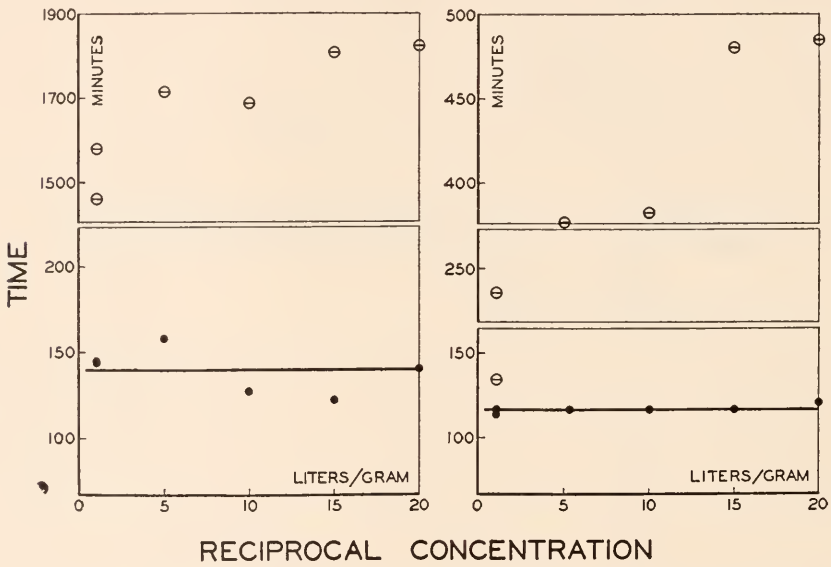

Fig. 3. Excystment of *Colpoda* at 20° C. after being blocked by very low oxygen tension. ●, time between admittance of air and emergence; ⊖, time between addition of excystment solution and emergence.

oxidative metabolism (Brown and Taylor, 1938). The fact that the reactions do not proceed in the second period in the absence of oxygen does not in itself prove that the normal limiting reaction is the oxidative metabolism—more refined experiments in which tests are made over a temperature range and in which excystment proceeds, but at a reduced rate due to oxygen tension being a limiting factor, are required.

Though the period dependent on extract concentration changes with temperature in the range 12° to 32° C. as though it were controlled by the time required for diffusion of a substance from the excystment solution, below 12° C. this period changes with temperature according

to the Arrhenius equation with a very high μ value (Brown and Taylor, 1938). From this, one might expect that a different process limits this period in the low temperature range. However, this other process, if it exists, is also independent of oxygen, for when the experiments were repeated at 11° C. it was found that the time after admittance of air for completion of excystment is at this temperature also independent of concentration and equal in duration to the period which is found by study of the relation between excystment time and concentration to be

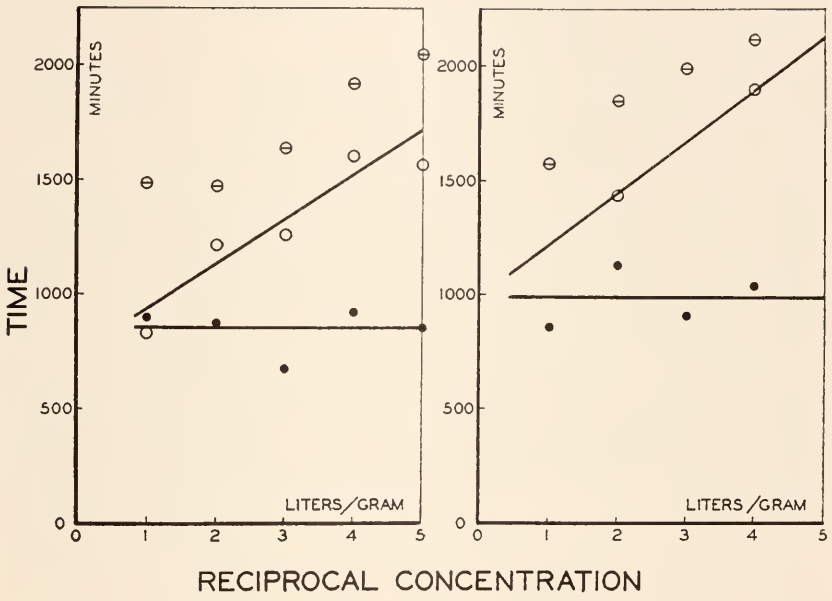

FIG. 4. Excystment of *Colpoda* at 11° C. after being blocked by very low oxygen tension. ●, time between admittance of air and emergence; ⊖, time between addition of excystment solution and emergence; ○, control-time between addition of excystment solution under aerobic conditions and emergence.

independent of concentration. Two of the low temperature series are shown in Figure 4. In the second of these series shown, the set of six dishes included four which were blocked by absence of oxygen and two to which the excystment solution was added at the time of admission of air; the conditions in the experimental and control dishes seem much more comparable in this case for it is seen that the extrapolated value for duration of the period independent of concentration coincides much more closely with the time for excystment after the block than in the cases in which the controls were run separately.

Discussion

At present four physiological periods in excystment of *Colpoda* have been sorted out by a quantitative study of excystment time under a variety of environmental conditions. Separation and characterization of the first and subsequent periods is by Brown and Taylor (1938) and this report and separation of the later periods is through the work of Taylor, Brown, and Strickland (1936) on the effects of x-ray irradiation at different stages of excystment.

The experimental characterization and physiological interpretation of these periods is briefly as follows:

I. A period whose duration is inversely proportional to the concentration of the organic constituents of the excystment solution (Brown and Taylor, 1938), and independent of oxygen tension (this report). Its duration changes with temperature as does the viscosity of the cytoplasm for a considerable temperature range (Brown and Taylor, 1938). This period is considered to be one during which diffusion of essential substances from the excystment solution takes place and possibly also an anaerobic reaction with high activation energy (Brown and Taylor, 1938, and this report).[3]

II + III + IV. A period whose duration is independent of the concentration of the excystment solution, and which is dependent upon oxygen (Brown and Taylor, 1938, and this report). The change in duration with change in temperature follows the Arrhenius equation with $\mu = 44{,}000$ calories/ mole below 15° C., 18,000 calories/mole between 15° and 25° C., and zero above 25° C. (Brown and Taylor, 1938). It is suggested that the value of $\mu = 18{,}000$ is associated with oxidative metabolism and $\mu = 44{,}000$ with an anabolic reaction of excystment (Brown and Taylor, 1938).

II. A period during which x-ray irradiation increases excystment time to the same extent as does irradiation at any time in Period I (Taylor, Brown, and Strickland, 1936).

III. A short period during which emergence from the cyst is prevented by the same x-ray dose that at other periods only delays excyst-

[3] A recent abstract of a paper by Danielli (1939) not yet published indicates that one might expect the rate of diffusion through living cell membranes to change with temperature according to the Arrhenius equation. This suggests that below 12° C. diffusion is the limiting factor in this first period in encystment rather than some postulated chemical reaction (Brown and Taylor, 1938) but that the mechanism limiting diffusion above 12° C. and below is different, i.e., a barrier of the type suggested by Danielli which requires diffusing molecules to possess greater than a certain kinetic energy in order to penetrate into the cell is limiting below 12° C., whereas cell structures that control diffusion rate according to their viscosity are limiting above 12° C.

ment or has no effect (Taylor, Brown, and Strickland, 1936). This period seems to be critical to the later building up of hydrostatic pressure which results in rupturing of the ectocyst membrane, for the irradiation prevents emergence but does not prevent the completion of differentiation of cilia or their functioning (unpublished observations of Taylor, Brown, and Strickland; see also Brown, 1938*a*).

IV. A period throughout which administration of an x-ray dose which caused a three-fold increase in excystment time if given during Periods I or II and prevented emergence if given in Period III has almost no influence on excystment time (Taylor, Brown, and Strickland, 1936). This period is considered separated from preceding ones by completion of a developmental reaction involving a substance which may be inactivated by x-ray irradiation during any preceding period.

That *Colpoda* blocked from excystment by absence of oxygen do not die or show any adverse effects for a block of at least 25 hours at 20° C. is opposite to the interpretation of some experiments with free-swimming *Colpoda* (Taylor and Strickland, 1938). In these experiments it was observed that free-swimming organisms die within a short time (97 per cent in two hours) in an unaerated dense bacterial suspension but do not die in a similar suspension which is aerated. From this it was concluded that low oxygen tensions cause death of free-swimming *Colpoda* within a few hours. This may indicate that certain enzyme systems which may be thrown out of balance by removal of oxygen and which then destroy the free-swimming organism are not activated until the second or later periods of excystment.

Summary

1. Excystment time is independent of oxygen tension down to 15 mm. Hg.

2. Excystment is blocked by very low oxygen tensions. This block is at a developmental stage between the excystment period dependent on concentration of the excystment solution and the periods independent of concentration.

3. The excystment process may be divided into four physiological periods characterized by the influence of temperature, concentration of the excystment solution, oxygen tension, and x-ray irradiation on the excystment time.

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